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Clinical Application of Plant Protease (Kimotab) in Surgical Field

by

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INTRODUCTION

Concerning the anti-inflammatory and anti-edema actvities of proteolytic euzymes, attention has been paid since the drop intravenous infusion of trypsin was made eight years ago. In that time, there were cases of allergy reaction appearing simultaneously with the temporary high fever. It seemed, therefore, that we had better refrain from dosing directly proteolytic enzyme to the patient.

Thereafter, with the progress of enzyme chemistry, α-chymotrypsin for injection was marketed and its clinical usefulness were ascertained as an anti-inflammatory agent.

Furthermore, in recent years, there appeared a plant protease, bromelain, and it was known that this protease had little toxicity. In other words, bromelain differs from chymotrypsin and trypsin in the manner of effects on fibrinogen and fibrin; in view of the point that bromelain gives no disturbance to the fibrinogen activity in the living body, it has been thought that bromalain would be an ideal proteolytic enzyme.

Having had the time of using KIMOTAB of MOCHIDA Pharmaceutical Co., containing bromelain as its main ingredient, the authors would report the basic experiments and clinical trials.

KIMOTAB contains :

50mg (20,000 units) bromelain and
1mg (2,500 units) Trypsin per tablet.

As to bromelain, main ingredient of Kimotab, it was found by MARBANO,[3] venezuelan chemist, in the fruit juice of Ananas Sativus in 1891. In the same year, CHITTENDEN[4] succeeded in separating crude bromelain from the juice by precipitation method using ammonium sulfate, sodium chloride and magnesium sulfate.

According to the explanation made by DIDISHEIM[5] and LEWIS in 1959, this enzyme had relatively weak activity on fibrinogen and reached the fibrin dissolving activity as large as 60 times as that of trypsin.

ANTI-CLOTTING ACTIVITY OF BROMELAIN

It was already reported by MARTIN, SMYTH and others[1] that there had been variations

Table 1 Effects on Blood Clotting Factors : Enzyme 10mg/kg I. V. Injection : Rabbit

Serum Prothrombin Time (seconds)

Time after enzyme admin. (minute)	0	30	60	90
Animal No.				
1	8.2		8.2	
2	8.1	10.4		10.8
3	8.0		9.5	
4	8.0	10.3		10.2
5	8.2	9.2	10.8	11.1
6	7.5	9.0	9.5	9.0
7	7.2	8.7	8.8	8.8
8	7.1	8.8		8.0
Average	7.8	9.4	9.36	9.65

Effect on Fibrinogen Concentration (mg/dl)

1	210		130	
2	250	200		140
3	220		80	
4	260	100		60
5	170	80	40	80
6	180	120	90	120
7	160	70	70	40
8	100	60	60	70
Average	194	105	78.3	85.0

Serum Plasmin concentration (Trypsin %)

3	39.3		41.4	
4	30.0	63.7		53.7
5	31.8	29.8	33.2	47.8
6	21.4	26.7	29.4	32.1
7	10.5	14.1	15.8	9.1
8	16.8	16.8	18.9	26.3
Average	24.97	30.22	28.14	33.08

in prothrombin value, antithrombin value and plasmin value when bromelain had orally been given to rabbits. The authors, however, examined the variations of blood clotting factors in case of intravenous injection of bromelain to rabbits and in case of oral administration of bromelain to dogs and human, on which report is made as follows:

(Methods of Experiment)

After administration of bromelain, blood was collected at regular intervals and was assayed according to the following method:

1. Prothrombin time : Lyoplastin-Mochida was used as thromboplastin, and the time was measured by means of Quick's one stage method.

2. Fibrinogen : Measurement was made by weight method.

3. Plasmin : Measurement was made according to heated plate method[2]. However, the plasmin value is shown by the percentage to the dissolved area produced by crystalline trypsin which is fixed at 100.

(Results of Experiment)

1. Rabbits

100 mg of bromelain (400 unit/mg) were dissolved in 10 ml of distilled water and the solution was used after eliminating insoluble matter by means of centrifugation. This solution was injected into the ear veins of rabbits. It was showed that plasmin level tended to the slight increase from 30 minutes after injection while fibrinogen showed the marked decrease after 30 minutes of injection maintaining the level for about an hour, as given in Table 1. In this quantity of injection there was seen no remarkable extension of prothrombin time.

The average values of each experiment was shown in the following figure.

2. Kimotab, compound of bromelain and trypsin, and its placebo were orally administered to dogs. Like rabbits, the examination was made on 7 dogs and the average values of respective results are graphed as shown below:

As shown on figure 2, in placebo group no change of plasmin level was seen while in Kimotab group there was seen $7 \sim 10\,\%$ increase of plasmin value. Con-

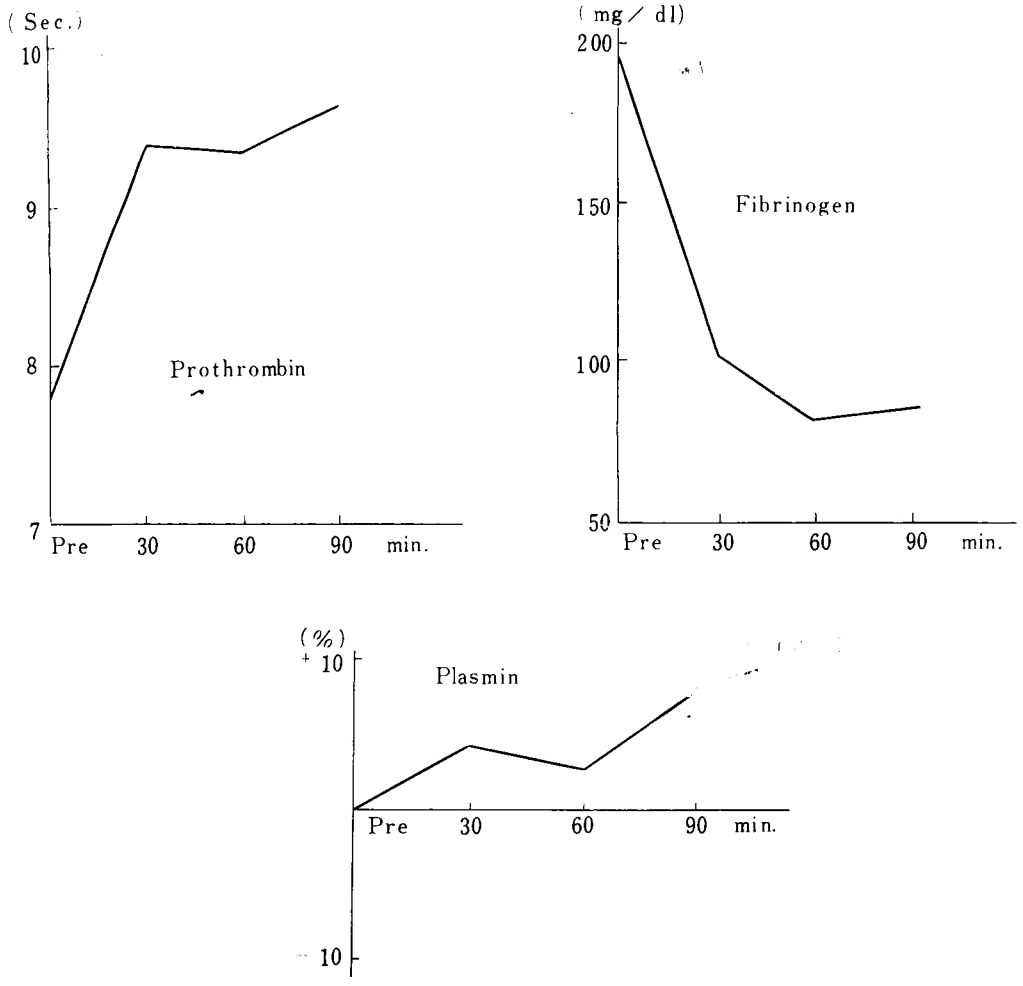

Fig. 1 Effects of Bromelain on Blood Clotting Factors. Rabbit. 10mg/kg Intravenous Injection

cerning fibrinogen, no significant difference was noticed between Kimotab group and placebo group. As regards prothrombin time, no difference was seen between both groups showing the variation within the normal ranges.

3. Human

To 20 cases of peripheral circulation disorder including spontaneous gangrene and thrombophlebitis, 8～10 tablets of Kimotab were administered to each patient daily for 10 days—14 days, and the measurement was made on the above blood factor during and after enzyme therapy. Plasmin value rose by 6～7% max. but it returned to the original level on the suspension of dosing. Fibrinogen showed 250～300 mg/dl, exceeding slightly the physiological variations and had no particular effect on clotting function. There was seen some extension of prothrombin time. (Fig. 3)

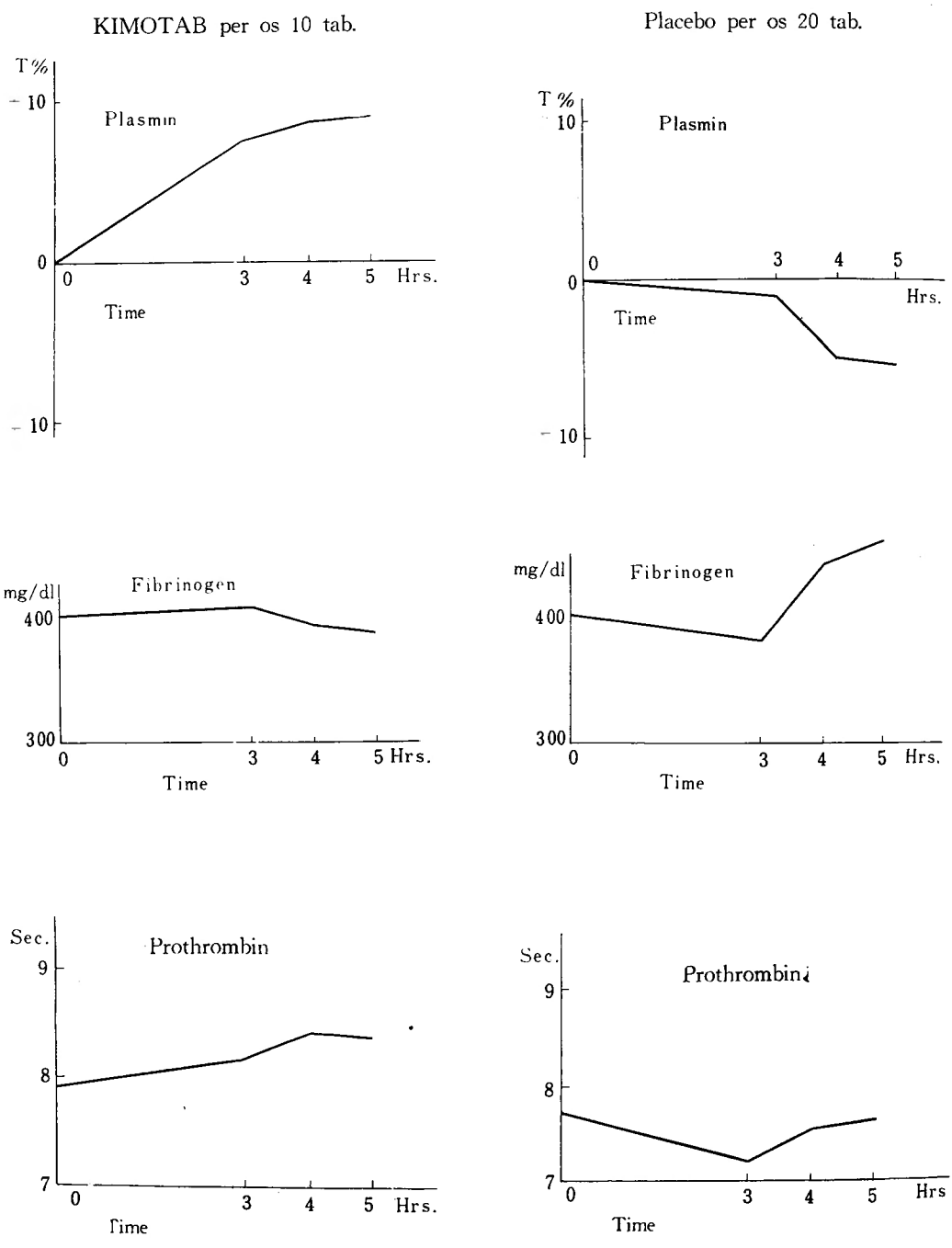

Fig. 2 Bromelain (Dogs)

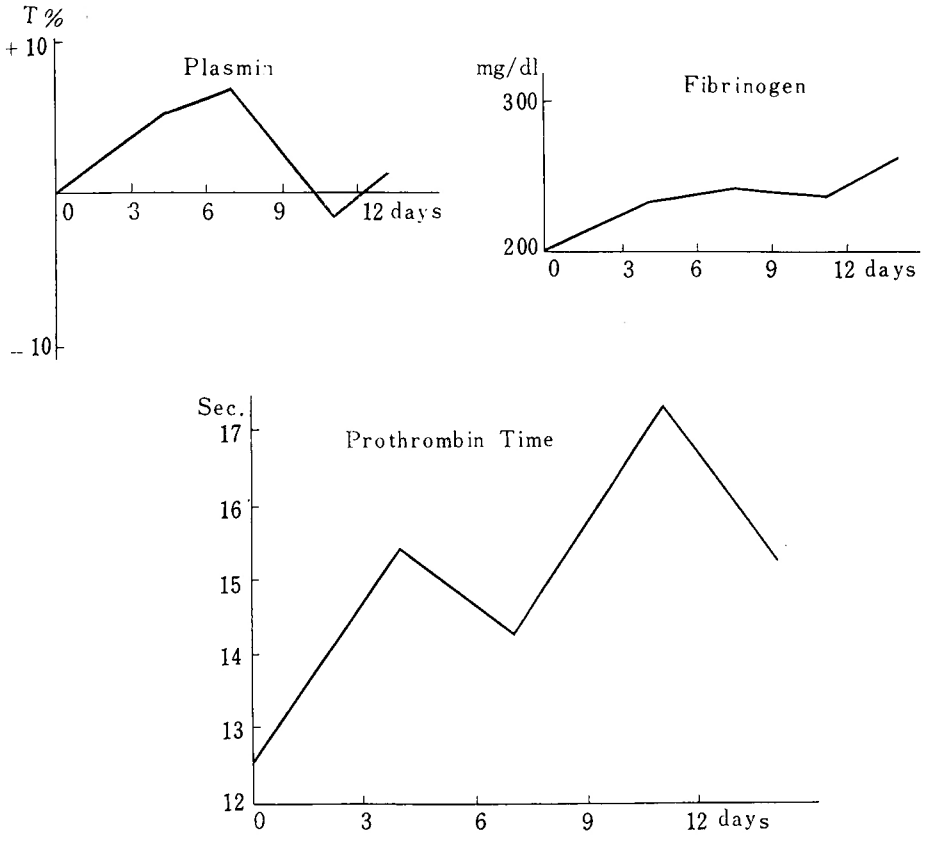

Fig. 3 Bromelain (Human)

RESULTS OF CLINICAL USE

(Inflammatory Diseases and Traumas)

Although the action mechanism of bromelain and trypsin, which are main ingredients of Kimotab, has not yet been made clear perfectly, it is presumed that these ingredients will be capable of having clinical effects with the improvement of permeability of inflammatory foci and edema parts as well as the restoration of peripheral circulation[6)7)].

First of all, we used Kimotab for the purpose of relieving the various symptoms caused by inflammatory diseases and traumas.

The subjects to whom Kimotab was administered were 25 in-patients and out-patients of Toho University Hospital (the 2nd Surgery) and were divided into 20 cases of inflammatory diseases and 5 cases of traumas. The ages of patients ranged from 12 to 67.

The dosages were min. 3 tablets of Kimotab per and max. 5 tablets per day, while the periods are 2 days at the the shortest and 10 days at the longest. Namely, the total quantities of Kimotab administered were 6 tablets to 50 tablets per patient.

The results are collectively shown on Table II and Table III. In inflammatory diseases excellent effect was found on 7 cases and good effect was found on 8 cases, but

Table 2 Inflammatory diseases

No.	Name	Age	Sex	Name of disease	Dosage (Tablets × Days)	Dose	Swelling	Pain	Fever	Edema
1	A	40	F	Arterial obstruction in the limbs	4×5	Before	−	++	−	−
						After	−	++	−	−
2	B	41	M	Gangrene	3×5	Before	−	−	−	−
						After	−	−	−	−
3	C	49	M	Gangrene	4×5	Before	−	++	−	−
						After	−	++	−	−
4	D	65	M	Thrombosis in left leg	3×5	Before	+	−	−	+
						After	±	−	−	±
5	E	20	M	Gangrene on right leg	3×5	Before	−	−	−	−
						After	−	−	−	−
6	F	67	F	Thrombo phlebitis	4×8	Before	++	±	−	±
						After	±	−	−	−
7	G	46	M	Gangrene	3×5	Before	−	+	−	−
						After	−	−	−	−
8	H	36	F	Thrombosis in legs	3×10	Before	+	−	−	+
						After	−	−	−	−
9	I	21	M	Gangrene	5×10	Before	−	+	−	−
						After	−	−	−	−
10	J	36	F	Thrombosis in legs	5×10	Before	+	−	−	+
						After	±	−	−	−
11	K	37	F	Thrombo phlebitis in legs	5×10	Before	++	+	++	++
						After	±	−	−	−
12	L	27	F	Whitlow on 2nd finger	4×3	Before	+	−	+	−
						After	+	−	+	−
13	M	16	F	Whitlow	4×3	Before	++	+	−	−
						After	+	+	−	−
14	N	44	F	Tubercular arthritis	3×5	Before	+	+	−	+
						After	+	−	−	−
15	O	46	F	Brachial phlegmone	3×3	Before	++	+	+	+
						After	−	−	−	−
16	P	12	F	Furuncle	4×7	Before	++	++	−	+
						After	−	−	−	−
17	Q	20	M	Phlegmone on outside of right knee	3×2	Before	++	+	+	+
						After	−	−	−	−
18	R	26	M	Periostitis of middle finger	3×5	Before	+	−	−	+
						After	−	−	−	−
19	S	20	M	After extirpation of ganglion	3×3	Before	+	−	−	+
						After	−	−	−	−
20	T	27	M	Swelling by leakage of hypertonic dextrose	3×3	Before	±	−	−	−
						After	±	−	−	−

Table 2 (Continued)

Hematoma	Hot feeling on part	Cold feeling on part	Secretion of pus	Formation of ulcer	Fibrinolysis	Venus anger	Others	Effect	Side-effect	Medicine used in combination
−	−	−	++	++				Non-effective	−	
−	−	−	++	++						
−	−	+	−	−	+			〃	−	
−	−	+	−	−	++					
−	−	++	−	−				〃	−	
−	−	++	−	−						
−	−	−	−	−	+	+		Good	−	
−	−	−	−	−	+	±				
−	−	+	+	+	+			〃	−	
−	−	+	−	−	+					
−	−	−	−	−				〃	−	
−	−	−	−	−						
−	−	+	−	−	+			〃	−	
−	−	+	−	−	+					
−	−	−	−	−	++	+		〃	−	
−	−	−	−	−	+	−				
−	−	+	+	+	+			〃	−	
−	−	+	−	−	++					
−	−	−	−	−	+	+		〃	−	
−	−	−	−	−	+	−				
−	++	−	−	−		++		Excellent	−	Chloramphenicol
−	−	−	−	−		−				
−	+	−	−	−				Non-effective	−	
−	+	−	−	−						
−	+	−	−	−				〃	−	
−	+	−	−	−						
−	+	−	−	−				Good	−	Bromsalicylate Streptomycin
−	−	−	−	−						
−	+	−	−	−		++	Solidification	Excellent	−	Sinomin
−	−	−	−	−		+				
−	++	−	++	+				〃	−	
−	−	−	−	−						
−	+	++	−	−				〃	−	
−	−	±	−	−						
−	+	−	−	−		+	Periosteum thicking	〃	−	
−	−	−	−	−		+				
−	−	−	−	−				〃	−	
−	−	−	−	−						
−	±	−	−	−				〃	−	
−	−	−	−	−						

Table 3 Traumas

No.	Name	Age	Sex	Name of disease	Dosage (Tablets × Days)	Dose	Swelling	Pain	Fever	Edema
1	A A	23	M	Electro-attack wound	4×3	Before After	+ +	+ 卄	− −	+ 卄
2	B B	21	M	Bruise on right leg back	4×5	Before After	卄 −	卄 −	+ −	+ −
3	C C	48	M	Compound fracturein left leg	3×7	Before After	卅 −	卄 ±	+ −	卄 −
4	D D	26	M	Bruise and sprain on legs	3×5	Before After	卄 −	+ −	+ −	+ −
5	E E	32	F	Peeling wounds and sprain on lower leg	3×4	Before After	卄 ±	+ −	± −	+ −

5 cases remained unchanged. In traumas excellent effect was found on 4 cases, but 1 case remained unchanged. Therefore, the general effectiveness registered 76%.

Concerning the symptoms of inflammation, of 18 cases of swelling, excellent effect was found on 11 cases and good effect was found on 4 cases as shown on Table IV; the effectiveness registered 85%. Also, high percentages of effectiveness were shown on other main symptoms, i. e. pain, fever, edema and hematoma.

About the 7 cases of inflammation on which Kimotab showed excellent effect, improvement was observed in 2～3 days after administration, and in particular it seemed that Kimotab had been effective relieving pain and swelling rapidly.

It seemed, however, that the perfect cure had not been expectable as to the inflammatory cases with chronic gangrene or thrombosis since long years ago. But, high percentages of effectiveness were in general registered to other acute inflammatory conditions including the cases where an antibiotic was used jointly. In this connection, it cannot be overlooked that Kimotab makes it easy for the antibiotic to penetrate into the focus in

Table 4 Effects Classified by Symptoms

Symptoms	No. of example	Excellent	Good	Fair	Noneffective	Effectivity
Swelling	18	11	4		3	83.3%
Pain	19	4	10	1	4	73.7%
Fever	8	1	5	1	1	75.0%
Hematoma	1	1				100.0%
Edema	16	2	11	2	1	81.3%
Hot feeling	12	2	6	1	3	66.7%
Cold feeling	6	1			5	16.7%
Formation of ulcer	5		3		2	60.0%
Secretion of pus	6	2	2		2	66.7%
Total	91	24	41	5	21	
		26%	45%	6%	23%	

Table 3 (Continued)

Hema-toma	Hot feeling on part	Cold feeling on part	Secretion of pus	Forma-tion of ulcer	Fibrino-lysis	Venous anger	Others	Effect	Side-effect	Medicine used in combinatoin
−	+	−	−	−				Non-effective	−	Chloramphenicol
−	+	−	+	+						
−	+	−	−	−				Excellent	−	
−	−	−	−	−						
++	+	−	−	−		++	Secretion of serum liquids	〃	−	Chloramphenicol
−	−	−	−	−		−				
−	−	−	++	−				〃	−	Francetin T. Powder
−	−	−	±	−						
−	−	−	−	−				〃	−	Sulfa drugs Francetin T. Powder
−	−	−	−	−						

addition to the anti-inflammatory and anti-edema activities of Kimotab.

Also, 4 cases out of 5 cases with trauma, relatively rapid relief in pain, swelling and hematoma was seen, while no effect was found in 1 case with electro-attack wound.

(Hemorrhoid Diseases)

The efficacy of drugs must be considered from many points of view. Particularly in case of making judgement based on subjective symptoms or external symptoms, the judgement is apt to be influenced by the subjectivities of patients and physicians, so it is difficult to make objective evaluation on the effects of medicine.

Therefore, the authors made strict examination by the so-called double blind method using placebo.

From hemorrhoid patients 30 cases with swelling after operation were selected, and the comparative examinations were made by dividing at random the said 30 examples into Kimotab group (20 cases) and placebo group (10 cases).

Concerning the dosages and periods, 8 tablets of Kimotab and placebo were administered at the first day and thereafter 4 tablets were given daily for 4～6 days.

For Kimotab group, 1 g streptomycin and 1 g mycilline intramuscular injections were made, while for placebo group 1 g streptomycin intramuscular injections were made to 2 cases and 1 g mycilline intramuscular injections were made to 8 cases. Besides the said intramuscular injections, cold packs were applied to diseased part.

The results are shown on Table V and Table VI. In Kimotab group, on the 3rd day the considerable relief of each symptom was seen for about a half of the cases and on the 5th day almost all of symptoms were disappeared. On the other hand, in placebo group no improvement was seen even on the 5th day.

Table VII shows the progress of severity of main complaint in Kimotab group. Similar effects were perceived on both pain and swelling regardless of their extents.

The comprehensive results are collectively shown on Table X, from which it is apear that there are remarkable difference in the effects between Kimotab group and placebo group.

Table 5 Kimotab Group

	Swelling	Pain	Hemorrhage	Swelling and pain	Pain and hemorrhage	Itching
1st day	20	20	15	20	20	18
2nd day	16	13	13	16	13	2
3rd day	11	9	8	10	7	1
4th day	5	8	4	5	4	0
5th day	0	1	1	1	1	0

Table 6 Placebo Group

	Sweelling	Pain	Hemorrhage	Itching
1st day	7	6	2	5
2nd day	7	6	2	5
3rd day	7	6	2	4
4th day	7	6	0	4
5th day	7	6	0	4

Table 7 Kimotab Group

		1st day	2nd day	3rd day	4th day	5th day
Pain	+	4	5	6	5	1
	++	9	6	2	0	0
	+++	7	5	3	0	0
Swelling	+	5	6	8	1	0
	++	8	5	1	0	0
	+++	7	4	0	0	0

Table 8 Kimotab Group

No.	Name	Age	Sex	Name of disease	Dosage (tablets)	Effect	Side-effect
1	A. B.	36	M	Fistula anni	20	Excellent	Nil
2	B. C.	24	F	Complete complicated fistula anni	24	Noneffective	〃
3	C. D.	25	M	Internal hemorrhoids	24	Good	〃
4	D. E.	32	M	Thrombosis inflammatory external hemorrhoids	24	〃	〃
5	E. F.	32	N	Internal hemorrhoids	20	Excellent	〃
6	F. G.	25	G	Internal hemorrhoids	28	Good	〃
7	G. H.	24	M	Fistula anni	20	〃	〃
8	H. I.	22	M	Complicated fistula anni	24	〃	〃
9	I. J.	25	M	Incarcerated hemorrhoids	20	Excellent	〃
10	J. K.	36	M	Internal hemorrhoids	24	Good	〃
11	K. L.	22	M	Internal hemorrhoids	24	〃	〃
12	L. M.	22	M	External hemorrhoids	24	〃	〃
13	M. N.	31	M	Incomplete external hemorrhoids	24	〃	〃
14	N. O.	32	M	Internal homorrhoids	24	〃	〃
15	O. P.	20	M	Internal and external hemorrhoids	24	〃	〃
16	P Q.	20	F	Internal hemorrhoids	24	〃	〃
17	Q. R.	50	M	Periproctol abscess	28	〃	〃
18	R. S.	38	M	Internal hemorrhoids	20	Excellent	〃
19	S. T.	29	M	Incarcerated hemorrhoids	20	Good	〃
20	T. U.	21	M	Internal and external hemorrhoids	24	〃	〃

Table 9 Placebo Group

No.	Name	Age	Sex	Name of disease	Dosage (tablets)	Effect	Side-effect
1	A. A.	35	M	Internal and external hemorrhoids	20	Non-effective	Nil
2	B. B.	52	M	Internal and external hemorrhoids	20	〃	〃
3	C. C.	22	F	Internal hemorrhoids	24	〃	〃
4	D. D.	52	M	Internal hemorrhoids	24	〃	〃
5	E. E.	32	M	Internal and external hemorrhoids	20	〃	〃
6	F. F.	32	M	Internal hemorrhoids	24	〃	〃
7	G. G.	27	M	Internal hemorrhoids	24	〃	〃
8	H. H.	27	M	Internal and external hemorrhoids	20	〃	〃
9	I. I.	32	F	Internal and external hemorrhoids	24	〃	〃
10	J. J.	30	M	Internal hemorrhoids	24	〃	〃

Table 10

	Excellent & Good*	Non-effective	Total
Kimotab group	19	1	20
Placebo group	0	10	10

*Excellent : Swelling and pain were remarkably improved on the 2nd day of treatment.
Good : Swelling was resolved and pain was removed on the 5th day of treatment.
Non-effective : No improvements were seen even on the 5th day of treatment.

CONCLUSION

As the basic experiments, the authors examined the anticlotting activities of bromelain on rabbits, dogs and human. And further, as the clinical experiments, examinations were made on the effects of Kimotab on inflammatory diseases, traumas and hemorrhoids. Concerning the hemorrhoids, double blind method was employed and the effects were judged based on the objective judgment.

These results are summarized as follows :

1. Plasmin level showed some increase after administering Kimotab to rabbits, dogs and human.

2. Prothrombin time and fibrinogen value did not vary respectively exceeding the ranges of physiological variation.

3. Concerning the 20 cases of inflammatory diseases, excellent result was obtained on 7 cases and good result was obtained on 8 examples, but 5 cases remained unchanged. As regards 5 cases of traumas, excellent effect was found in 4 examples, but 1 remained unchanged. As to 20 cases of hemorrhoid diseases, 4 excellent results and 15 good results were obtained, and no effect was found only in one case.

4. Speaking of the cases on which excellent effect was found, these mean that the effect was obvious in 2—3 days after administrating Kimotab to the patients. Particularly, Kimotab was effective on relieving swelling, pain and edema. Also, it is worthy of notice that there were cases on which remarkable effect were found by solely dosing Kimotab to the patients with inflammatory diseases.

It was proved by double blind method that Kimotab was remarkably effective on

swelling and pain after operation of hemorrhoid diseases in particular.

5. The effect of Kimotab was unexpectable on chronic gangrene and arterial obstruction.

6. Kimotab being entero-soluble tablets, it was easy to adjust the dose, while no such side-effects as allergy reaction and so forth were observed during clinical experiments.

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和文抄録

外科領域に於ける植物性蛋白分解酵素ブロメラインの使用経験

東邦大学医学部第2外科（主任 粟津三郎教授）

長山 寛・杉山卓乗・多田隆信
岡田价弘

植物性蛋白分解酵素ブロメラインと動物性蛋白分解酵素トリプシンの合剤であるキモタブを外科領域の各種疾患に対し，抗浮腫，抗炎症の目的で使用し，特に痔疾患術後の炎症に対しては，プラセボを用いたDouble Blind 法によつてその効果を検討した．

また，ウサギ，犬および実際の患者血清について，2，3の凝血学的検索を行なつた．

その結果，

1. 本剤投与後の血中プラスミン活性は，増大の傾向が見られる．

2. フィブリノーゲン及びプロトロンビン時間は，正常範囲内の変動であつた．

3. 炎症性疾患20例中，15例，また外傷5例中4例に効果が認められた．痔核手術後の20例では，19例に有効であつたのに対し，プラセボ投与の10例では，全例無効であつた．

4. しかし，慢性の脱疽及び動脈閉塞性疾患には，全く効果が認められなかつた．